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Anti-giardial activity of gastrointestinal remedies of the Luo of East Africa

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Abstract

Activity in an in vitro assay with *Giardia lamblia* provided a test of the validity of a quantitative methodology used in an ethnobotanical survey of the Luo people of the Lake Victoria basin of Kenya and Tanzania. Forty-five taxa of remedies for gastrointestinal problems were reported by four or more independent informants and a log-linear model was used to calculate a statistical measure of informant consensus. Methanolic extracts of 21 of 36 taxa assayed were lethal or inhibited growth of *Giardia* trophozoites at 1000 ppm; 7 species were lethal at 500 ppm. Non-cathartic species are more likely to be active than cathartics. Lethal species of non-cathartics are reported by informants more frequently than non-lethal species although the lack of statistical significance did not provide satisfactory support for the validity of the quantitative methodology as a predictor of efficacious remedies.

Keywords: *Giardia lamblia*; Protozoa; Plants, Medicinal

1. Introduction

The usefulness of field surveys for identifying efficacious herbal medicines and potential plant sources of new pharmacologically active agents is limited by the anecdotal nature of the data obtained. The efficiency of medicinal plant screening would be increased by improved criteria to identify which plants are most likely to provide positive results. Quantitative methods of data collection and analysis have been proposed as a way to make the process of identifying candidates for assessment more objective (Johns et al., 1990; 1994) but have not been validated. Validation of folk remedies and of statistical methods of evaluating remedies can employ bioassays which have a rational rela-

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tionship to the folk medicinal use under consideration.

Parasitic infections are the cause of many gastrointestinal problems, and remedies for gastrointestinal conditions would be expected to be more effective against agents causing dysenteric symptoms than a random selection of folk medicines. Remedies used to treat diarrhoea and dysentery are more likely than cathartics to show activity against known agents causing infectious diarrhoea.

Giardia lamblia (G. duodenalis) with a worldwide distribution is a common human enteric protozoan pathogen, especially in children, and is an important cause of acute diarrhoea and nutrient malabsorption in developed and developing countries alike. Treatment failures can occur with all of the drugs commonly used to treat giardiasis (Boreham et al., 1984; 1987). Metronidazole, the current drug of choice, is carcinogenic in animals and has side effects that are particularly serious in children. Moreover, resistance to current drugs has been reported.

The availability of culture medium for the cultivation of *Giardia* trophozoites over the past 15 years (Meyer and Radulescu, 1984) has facilitated the development of in vitro assays for the evaluation of new drugs effective against *Giardia*. Several studies which have attempted to identify new drugs for use in giardiasis (Takeuchi et al., 1985; Boreham et al., 1987; Crouch et al., 1986; Farthing et al., 1987;) have considered only chemotherapeutic agents with known activity against protozoa, bacteria and other parasites. There are no reports in the literature on attempts to identify new classes of compounds derived from plants for the treatment of giardiasis.

As a validation procedure of our quantitive methodology we assayed 35 herbal remedies of the Luo of Kenya and Tanzania identified in a field survey for activity against *Giardia lamblia*. In turn the method attempts to identify new agents with anti-giardial activity.

2. Materials and methods

2.1. Field study

We previously reported the herbal remedies of the Luo of Siaya District, Kenya (Johns et al., 1990). The current investigation is based upon an extended field study employing the same interviewing methodology among Luo informants in Tarime and Musoma Districts, Mara Region, Tanzania (1990), Homa Bay District, Kenya (1992) and Migori District, Kenya (1993). Unless otherwise indicated in Table 1 herbarium specimens are

Table 1
Gastrointestinal remedies of the Luo listed alphabetically by genus

Plant species (Family) Herbarium specimens[a]	Part[b]	No.[c]	r_{ij}[d]
Albizia coriaria Oliv. (Fabaceae)	rt, bk	4	1.47
Aloe[e] spp. (Liliaceae)	rt	6	1.37
Aspilia pluriseta Schweinf. 93-212 NAI (Asteraceae)	lf	4	1.10
Capparis fascicularis DC. (Capparaceae)	rt	7	1.69
Carissa edulis[e] (Forsk.) Vahl (Apocynaceae)	rt	14	2.34
Cassia didymobotrya[e] Fres. (Fabaceae)	lf, rt	9	1.94
Cassia occidentalis[e] L. (Fabaceae)	rt, lf	12	2.35
Cassia hildebranditii[e] Vatke, C. siamea Lam. (Fabaceae)	lf, rt	11	2.28
Catharanthus roseus G. Don (Apocynaceae)	rt	5	1.48
Clerodendrum myricoides (Hochst.) Vatke 93-91 NAI (Verbenaceae)	rt	6	1.38
Coleus kilimandscharica Gurke (Lamiaceae)	lf	9	1.63
Combretum collinum Fres. (Combretaceae)	rt,	5	1.19
92-101 NAI; C. molle G. Don	lf		
93-173 NAI (Combretaceae)	lf		
Commiphora africana (A. Rich.) Engl. (Burseraceae)	rt	5	1.17
Crotalaria brevidens[e] Benth. (Fabaceae)	lf	4	1.03
Cucumis dipsaceus Spach 90-113 ITM, NAI (Cucurbitaceae)	fr	5	1.57
Euclea divinorum[e] Hiern 92-126, 92-144 NAI (Ebenaceae)	rt	33	3.25

Table 1 (continued)

Plant species (Family) Herbarium specimens[a]	Part[b]	No.[c]	τ_{ij}[d]
Gutenbergia cordifolia Benth. 92-19, 92-195, 92-210 NAI (Asteraceae)	lf	9	1.93
Harrisonia abyssinica Oliv. (Simaroubaceae)	rt	16	2.38
Justicia exigua 88-101 NAI, *Justicia betonica* 93-163 NAI (Acanthaceae)	pl	4	0.88
Kigelia africana (Lam.) Benth. (Bignoniaceae) Z	bk	8	1.80
Lannea schweinfurthii (Engl.) Engl. (Anacardiaceae)	bk, rt, lf	7	1.44
Leonotis nepetifolia R. Br. (Lamiaceae)	rt	9	2.05
Leucas calostachys Oliv. (Lamiaceae)	lf, rt	5	1.17
Maytenus senegalensis (Lam.) O. Kuntze 88-421, 96-116 NAI (Celastraceae)	rt	4	1.03
Melia azedarach[e] L. (Meliaceae)	lf	5	1.39
Microglossa pyrifolia (Lam.) O. Kuntze. (Asteraceae)	rt	6	1.51
Ocimum basilicum[e] L., O. suave Willd. (Lamiaceae)	lf	17	2.48
Ormocarpum trichocarpum (Taub.) Engl. (Fabaceae)	rt, lf	4	0.93
Ozoroa insignis Del. 93-137 NAI (Anacardiaceae)	rt	6	1.59
Rhoicissus revoilii Planch. (Vitaceae)	tb	6	1.59
Rhus natalensis[e] Krauss; R. vulgaris Meikle (Anacardiaceae)	rt	7	1.51
Schkuhria pinnata[e] (Lam.) O. Ktze. (Asteraceae)	pl	17	2.66
Solanum incanum L 88-186, 88-458, NAI; *Solanum nigrum* L. (Solanaceae)	rt, lf	6	1.58
Sonchus schweinfurthii Oliv. et Hiern (Asteraceae)	lf	5	1.41
Sorghum bicolor Pers. (Poaceae)	sd	4	1.10
Tithonia diversifolia (Hemsl.) Gray 90-80 ITM (Asteraceae)	lf	4	0.88
Toddalia asiatica (L.) Lam. 90-298 ITM, 92-105 NAI (Rutaceae)	rt	13	2.17
Tylosema fassoglensis[e] (Schweinf.) Torre et Hillc. (Fabaceae)	rt	5	1.15
Vernonia amygdalina[e] Del. (Asteraceae)	rt, lf	4	1.10
Vernonia glabra Vatke (Asteraceae)	rt, lf	6	1.63
Vernonia adoensis Walp. 92-150 NAI (Asteraceae)	rt	4	0.80
Warburgia salutaris (Bertol.f.) Chiov. (Canellaceae)	bk	6	1.63
Ximenia americana L.; *Ximenia caffra* Sond. 90-82 ITM, 92-86 NAI (Olacaceae)	rt	8	1.79
Zanthoxylum chalybeum Engl. (Rutaceae)	rt, sd	8	1.78

[a]NAI, Nairobi University Herbarium; ITM, Institute of Traditional Medicine, Dar es Salaam.
[b]bk, bark; lf, leaf; pl, plant; rt, root; sd, seed; tb, tuber.
[c]Number of independent reports.
[d]Interaction.
[e]Cathartic.

cited in Johns et al. (1990). All specimens were authenticated by S. Mathenge and J. Kokwaro of the Nairobi University Herbarium (NAI) and/or E.B. Mhoro, Institute of Traditional Medicine, Dar es Salaam (ITM).

The resulting Luo ethnomedicinal database contains information from 134 herbalists and comprises 2103 remedy reports on the use of 276 genera of plants. For the data analysis all reported diseases were grouped into 47 categories generally based on physiological systems. The current analysis focused on taxa used to treat a broad classification of gastrointestinal problems.

2.2. Quantitative criteria of consensus

The total number of independent reports of a particular remedy was employed as one measure of consensus.

However, the prevalence of a particular plant or disease determines the likelihood of encountering any particular remedy (defined as the use of a specific plant to treat a specific disease). Thus, there is greater probability of a common plant (common either in abundance or in its cultural value as a medicine) being reported to treat a common disease than a rare plant to treat a disease of limited occurrence.

To establish some measure of confirmation independent of the simple probability of encountering specific plants and diseases, we applied a log-linear model to our data (Johns et al., 1990). If n_{ij} is the total number of people who said that plant *i* is used to treat disease *j*, then we postulate that the n_{ij} is influenced by factors that are due to abundance of plant *i*, prevalence of disease *j* and the potential for plant *i* as a cure for disease *j*. The interaction of *i* and *j* (τ_{ij}), an indication of the potential of plant *i* as a cure for disease *j*, is of interest as a quantitative measure of the degree of confirmation of any particular remedy.

2.3. Sample collection and preparation

Samples of 36 gastrointestinal remedies identified in the field study were collected in Siaya District in April 1988, or in Tarime District in July-August 1990 and preserved fresh in methanol in nalgene plastic bottles. Samples were transported by air to Canada and stored at 4°C until extracts were concentrated to dryness by rotary evaporator. Crude methanolic extracts were stored at −20°C.

2.4. Description of parasite

Giardia lamblia strain WB (American Type Culture Collection #30957) was isolated from a patient with chronic symptomatic giardiasis (Smith et al., 1982) and belongs to the most common group of human isolates. In these studies, a clone of WB has been generated by limiting dilution (Campbell and Faubert, 1994).

2.4. In vitro assay of anti-giardial activity

Crude methanolic plant extracts were tested with a bioassay of the growth inhibition of *Giardia lamblia* trophozoites cultured in TY1-S-33 medium (Diamond et al., 1978). Concentrated extracts were diluted with dimethylsulfoxide (DMSO) and incorporated in the culture medium to a final concentration of DMSO in the medium of 0.2%. The medium was subsequently filtered with a sterilizing membrane of 0.22 μm. Extracts were tested in triplicate at 1000 and 500 ppm in 96 well microculture plates (Nunc Company). Each well contained 300 μl of medium and 50 000 trophozoites. Plates and controls (0 and 0.2% DMSO) were incubated at 37°C under anaerobic

Table 2
Effects of methanolic extracts of 36 Luo gastrointestinal remedies (ranked according to number of independent reports) on survivorship of *Giardia lamblia* in vitro

Plant	Part[a]	Activity[b] (ppm)	
		500	1000
Euclea divinorum[c]	rt, bk	–	–
Schkuhria pinnata[c]	pl	–	–
Ocimum suave[c]	lf	–	+
Harrisonia abyssinica	rt, bk	+++	+++
Carissa edulis[c]	rt, bk	–	–
Toddalia asiatica	rt, bk	+	+++
Cassia occidentalis[c]	lf	–	–
Cassia siamea[c]	lf	+	+++
	rt	–	+
Coleus kilimandscharica	lf	+	+++
Leonotis nepetifolia	rt	–	–
Cassia didymobotrya[c]	rt, bk	+	+
Ximenia caffra	rt, bk	+++	+++
Zanthoxylum chalybeum	rt, bk	–	–
Lannea schweinfurthii	rt, bk	n/a[d]	+++
Rhus natalensis[c]	rt, bk	+	+++
Rhoicissus revoilii	tb	–	–
Ozoroa insignis	rt, bk	+++	+++
Microglossa pyrifolia	rt, bk	+++	+++
Vernonia glabra	rt	n/a	+
Aloe sp.[c]	rt	–	–
Warburgia salutaris	bk	–	–
Sonchus schweinfurthii	lf	+++	+++
Solanum incanum	rt, bk	n/a	+++
Solanum nigrum	lf	+++	+++
Commiphora africana	rt, bk	+	+++
Catharanthus roseus	rt	–	+
Melia azedarach[c]	lf	–	–
Vernonia sp.	rt, bk	+	+++
Albizia coriaria	rt, bk	+++	+++
Crotalaria brevidens[c]	lf	–	–
Ormocarpum trichocarpum	rt, bk	–	–
Vernonia amygdalina[c]	lf	–	–
Gynandropsis gynandra[c]	lf	–	–
Acanthus arborea	rt, bk	–	–
Psidia arabica	rt	–	+++
Celosia schweinfurthiana	pl	–	–

[a]bk, bark; lf, leaf; pl, plant; rt, root; tb, tuber.
[b]+++, 100% kill; +, growth inhibition; –, no effect.
[c]Cathartic.
[d]Sample not analyzed.

conditions and the growth of the parasite determined by counting the number of trophozoites present in the culture after 72 h of incubation. Metronidazole was used as a positive control in the assay.

3. Results

Remedies confirmed independently by four or more Luo informants to treat general stomach and intestinal complaints including stomachache, diarrhoea and constipation are listed in Table 1. Anthelmintics are excluded from the compilation. Also reported are the total number of times plants were reported as gastrointestinal remedies and the interaction (τ_{ij}). Cathartic species are specifically indicated in Table 1. The majority of informants described remedies as for stomach problems rather than specifically as cathartics or anti-diarrhoeal agents. Cathartics are identified as those species with use against constipation which were not also reported as anti-diarrhoeal, or those for which the number of reports of cathartic use was at least double the reports as anti-diarrhoeals.

Anti-giardial activities of 36 gastrointestinal remedies at 1000 and 500 ppm appear in Table 2. The table contains data on 3 species that do not appear in Table 1. Two species reported by less than 3 informants are *Acanthus arborea* Forsk. (2 reports) and *Psiadia arabica* Jaub et Spach. (1 report). *Celosia schweinfurthiana* Schinz. which is used with high consistency (7 reports) as a treatment for gastrointestinal worms did not show activity against *Giardia*.

Table 4
Differences in proportions of cathartics and non-cathartics lethal to *Giardia*

	Lethal	Non-lethal	Totals
(a) 1000 ppm			
Cathartics	1	12	13
Non-cathartics	14	8	22
Totals	15	20	35
Pearson χ-square $P < 0.01$			
(b) 500 ppm			
Cathartics	1	12	13
Non-cathartics	7	12	19
Totals	8	24	32
Pearson χ-square $P = 0.07$			

4. Discussion

Fifteen of the 36 remedies tested (plus leaves of *Cassia siamea*) were lethal to *Giardia* at 1000 ppm and an additional 5 species inhibited growth at this concentration (Table 2). Seven of 35 species were lethal at 500 ppm and 7 inhibited growth. To test the hypothesis that those remedies encountered the most frequently in the survey are more likely to be efficacious, the mean number of reports of the species that are lethal and non-lethal at each of 1000 or 500 ppm were compared (Table 3). We predict that lethal species would be reported more frequently than non-lethal ones. Comparison of the mean interaction (τ_{ij}) between lethal and non-lethal species provides a further test of the hypothesis.

Table 3
Comparison of mean consensus value between lethal and non-lethal species

	1000 ppm		500 ppm	
	Lethal	Non-lethal	Lethal	Non-lethal
All remedies				
Mean number of reports ± S.D.	6.93 ± 3.79	9.05 ± 7.15	7.25 ± 3.73	8.52 ± 6.73
Mean interaction	1.48 ± 0.55	1.79 ± 0.63	1.66 ± 0.31	1.68 ± 0.69
Non-cathartics				
Mean number of reports ± S.D.	6.92 ± 3.95	5.75 ± 2.19	7.29 ± 4.03	6.00 ± 3.19
Mean interaction	1.48 ± 0.57	1.51 ± 0.38	1.68 ± 0.33	1.40 ± 0.57

Contrary to prediction when all remedies are considered the non-lethal species have higher values for mean number of reports or τ_{ij} than lethal ones. However, as illustrated in Table 4 there is a greater likelihood that cathartics are non-lethal in comparison to non-cathartics (Pearson χ-square, $P < 0.01$). Thus, when cathartics are removed from the analysis of the means (Table 3), the lethal species have higher values for mean number of reports than non-lethal species at both 500 and 1000 ppm (7.29 ± 4.04 vs. 6.00 ± 3.19 respectively for the assay at 500 ppm). At 500 ppm the value of τ_{ij} is also higher for lethal species (1.68 ± 0.33 vs. 1.40 ± 0.57). Because of the high variability in both categories both the parametric Student's *t*-test and non-parametric Wilcoxon rank test do not demonstrate statistical significance of the results.

With 35 species assayed the sample size is relatively small. Assay of the remainder of the Luo gastrointestinal remedies might provide a satisfactory validation of the quantitative methodology.

The clear effect of removing known cathartics from the data analysis demonstrates that with a multi-factored general ailment like gastrointestinal disorder, it is necessary to determine where possible what specifically is being treated. This might be accomplished with a survey involving a larger number of informants or with a more targeted questionnaire. The large survey that might be necessary to obtain this information does limit the general usefulness of the method.

Only one assay was used for the validation of the quantitative methodology and some plant species with activity in controlling gastrointestinal infections may have been missed. For example, it is possible that some of the plant remedies act by inhibiting gastrointestinal motility rather than directly upon parasites. Moreover, giardiasis is only one of a number of common causes of infectious diarrhoea and plants that were not active against *Giardia* could be assayed against other pathogens such as bacteria, viruses and other protozoa such as *Entamoeba histolytica*.

The analysis to date suggests that the log-linear model is not an improvement over simply using the number of independent reports of a remedy as an indicator of its likely efficacy relative to other remedies.

5. Conclusions

The quantitative method employed in data collection and analysis shows promise as a method for predicting which remedies are likely to have anti-giardial activity. Those with the highest number of independent reports such as *Harrisonia abyssinica* and *Toddalia asiatica* are more likely to show activity. Our database contains 145 taxa that are identified by at least one informant as used to treat gastrointestinal problems. Few of these would be expected to have activity worthy of further investigation and the identification of those employed by traditional healers with the greatest degree of consensus provides a method of prioritizing laboratory analysis. However, the method needs further refinement and testing to establish satisfactorily statistical support of its validity.

Based on our bioassay results the 7 species with lethal activity at 500 ppm (Table 2) are good candidates for further investigation as control agents against *Giardia* or other protozoan parasites.

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